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Source: Avian Diseases, 47(3):618-626. 2003.
Published By: American Association of Avian Pathologists
DOI: 10.1637/6091
URL: http://www.bioone.org/doi/full/10.1637/6091

AVIAN DISEASES 47:618–626, 2003

The Effects of Avian Leukosis Virus Subgroup J on Broiler Chicken Performance and Response to Vaccination

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Received 6 November 2002

SUMMARY. The effects of avian leukosis virus subgroup J (ALV-J) infection on meat-type chickens reared in a simulated commercial setting were evaluated. Each of three ALV-J isolates was evaluated with both simulated horizontal transmission (SHT) and simulated vertical transmission (SVT). Mortality, morbidity, disease condemnations, and feed conversions were increased and body weights at processing were decreased in ALV-J infected birds as compared to sham inoculated hatch mates. The adverse effects of ALV-J infection were more severe in birds exposed by SVT than in birds exposed by SHT.

At 8 weeks of age response to vaccination for infectious bronchitis virus and Newcastle disease virus or prior exposure to a pathogenic reovirus was assessed in the ALV-J and sham inoculated broiler chickens by challenge studies. Although not statistically significant, an overall trend of decreased protection to challenge after vaccination, or prior exposure, was observed in the ALV-J inoculates as compared to sham inoculated hatch mates. Differences in vaccine response were most evident in groups inoculated with ALV-J by the SVT route.

RESUMEN. Efectos del virus del subgrupo J de leucosis aviar sobre los rendimientos de los pollos de engorde y su respuesta a la vacunación.

Se evaluaron los efectos de la infección con el virus del subgrupo J de leucosis aviar en aves de la línea pesada, mantenidas bajo condiciones similares a las de crianza comercial. Cada una de tres cepas del virus J de leucosis fueron evaluadas bajo condiciones simuladas de transmisión horizontal y vertical. La mortalidad, morbilidad, descartes en el matadero y conversiones alimenticias estuvieron aumentadas, mientras que los pesos corporales obtenidos en el matadero fueron menores en las aves infectadas con el virus del subgrupo J, comparado con los controles. Los efectos adversos de la infección con el virus J fueron más severos en las aves expuestas por medio de la transmisión vertical que las expuestas por transmisión horizontal. La respuesta a la vacunación contra bronquitis infecciosa y la enfermedad de Newcastle o la exposición previa a un reovirus patógeno fue evaluada a las 8 semanas de edad mediante desafíos. Aunque no se encontraron diferencias estadísticamente significantes, se observó una tendencia general de menor protección al desafío después de la vacunación, o a la exposición previa, en las aves inoculadas con el virus J de leucosis comparada con las aves controles. Las diferencias en la respuesta vacunal fueron más evidentes en los grupos inoculados con el virus J de leucosis por la vía vertical.

Key words: Avian leukosis virus subgroup J, broiler chickens, commercial poultry

Abbreviations: ALV-J = avian leukosis virus subgroup J; CAV = chicken anemia virus; CEF = chicken embryo fibroblast; CID_{50} = mean chicken infective dose; EID_{50} = mean embryo infective dose; ELISA = enzyme-linked immunosorbent assay; HVT = herpesvirus of turkeys; IA = intraabdominal; IBDV = infectious bursal disease virus; IBV = infectious bronchitis virus; MDV = Marek's disease virus; NDV = Newcastle disease virus; PCR = polymerase chain reaction; PFU = plaque-forming units; PI = postinfection; SHT = simulated horizontal transmission; SPF = specific-pathogen free; SVT = simulated vertical transmission; $TCID_{50}$ = mean tissue culture infective dose

Avian leukosis virus subgroup J (ALV-J) is a retrovirus that causes an economically important disease in meat-type chickens. ALV-J was first recognized in the late 1980s (5) and subsequently became a disease concern for the poultry industry worldwide. Eradication programs have been enacted in recent years to control ALV-J by removing infected breeding stock from flocks, thereby reducing virus spread by vertical transmission.

Economic losses from ALV-J are often due to mortality in meat-type breeder chickens from neoplastic disease. The most common tumor type associated with ALV-J is myeloid leukosis, although a wide variety of tumors have been associated with the virus, including nephroblastomas, hemangiomas, and histiocytic sarcomatosis (1,5,6). Nonneoplastic disease caused by ALV-J is less well defined; however, body weight suppression (11) and a poor response to environmental stressors have been observed in ALV-J infected chickens (pers. obs.). In general, the type and severity of disease that develops is believed to be influenced by age at exposure, environmental stressors, host genetics, and the pathotype of the ALV-J isolate present.

Previously the economic consequences of ALV-J infection of meat-type chickens were expected to be minimal, since broilers are slaughtered before the age (7 to 12 weeks) at which ALV-J induced neoplasia, which would affect livability or condemnations, often develops (6). However, observations of ALV-J infected meat-type chickens in commercial operations suggest that ALV-J induced nonneoplastic disease may have an economically significant adverse impact on performance.

This study was performed to more clearly define the pathogenesis of ALV-J in meat-type chickens reared in a simulated commercial environment. The role of exposure at different ages by simulating horizontal and vertical transmission and the role of breed/ALV-J isolate combinations in disease development were also evaluated. Additionally, vaccine response was assessed in ALV-J and sham inoculated chickens as a preliminary *in vivo* evaluation of potential immunosuppression related to ALV-J exposure in a commercial setting.

MATERIALS AND METHODS

Virus. The ADOL-Hc1 isolate (4) was provided by Dr. Aly Fadly (USDA-ARS, Avian Disease and Oncology Lab (ADOL), East Lansing, MI). The UD-3 and UD-4 isolates were obtained from the buffy coats of ALV-J infected commercially produced chickens. The isolates were propagated by no more than three passages in C/E (Kestrel, Waukee, IA) chick embryo fibroblast (CEF) cell cultures. CEF cell cultures were prepared by standard techniques (10) with the addition of 2 μg/ml of hexadimethrine bromide (polybrene) (Sigma, St. Louis, MO) to the growth medium immediately prior to virus inoculation.

Passage of ALV-J in C/E CEF cell cultures was accomplished by inoculating the virus onto CEF cell culture monolayers and incubating at 37 C, 5% CO_2 for 6 to 7 days, at which time the CEF cell cultures were subcultured and incubated for an additional 6 days. Supernatants were harvested and used as inocula.

Virus isolation. Whole blood was collected in an equal volume of Alsever's solution and centrifuged at 1,000 × *g* for 5 min. Approximately 200–250 μl of buffy coat was collected in 96 well plates and stored at −80 C. Buffy coats were subjected to one or two freeze thaw cycles prior to virus isolation.

Buffy coats were thawed and 25 μl added to 3.5×10^5 cells/ml of C/E CEFs in 175 μl of media per well in a 96 well plate. Plates were incubated for 7 to 8 days at 37 C, 5% CO_2. The cell culture plates were then exposed to two freeze/thaw cycles and tested for the presence of the viral p27 protein by commercial enzyme-linked immunosorbent assay (ELISA) (Idexx, Westbrook, ME).

Experimental chickens. Unincubated broiler hatching eggs were obtained from commercial sources and set to hatch, except for the ADOL-Hc1 SHT study where day-old chicks were obtained from a commercial hatchery. Breeder flocks that were the source of the experimental chickens were screened for the presence of ALV-J viremia by virus isolation prior to obtaining eggs or chicks as previously described.

The UD-3 and UD-4 isolates were evaluated in chickens from their respective genetic lines of origin. ADOL-Hc1 was evaluated in a third genetic line, unrelated to the line of the origin of the isolate. Chickens for both UD-4 experiments were progeny from the same breeder flocks.

Simulated horizontal transmission (SHT) studies. Three ALV-J isolates were evaluated by SHT: ADOL-Hc1, UD-3, and UD-4. At 18 days of incubation all embryos were vaccinated *in ovo* for Marek's disease with 4000 plaque-forming units (PFU) of herpes virus of turkey (HVT), 1800 PFU of SB-1 (Merial, Gainesville, GA), and Rispens strain (Intervet, Inc., Millsboro, DE). At hatch all chicks were vaccinated by aerosol, with a commercial spray cabinet, for Arkansas (Ark) (Intervet Inc.) and Massachusetts (Mass) type infectious bronchitis virus (IBV) (Merial) and B1 type Newcastle disease virus (NDV) (Merial). Approximately $10^{2.6}$ mean embryo infective dose (EID_{50}), $10^{3.5}$ EID_{50}, and $10^{1.9}$ EID_{50} of Ark IBV, Mass IBV, and NDV vaccine were administered per chick respectively. The birds were revaccinated at 2

weeks of age with homologous respiratory vaccine via drinking water (approximately $10^{4.6}$ EID_{50} of IBV and $10^{6.5}$ EID_{50} of NDV vaccine) (Schering-Plough, Millsboro, DE).

At hatch the chickens were divided into two equal groups of 600 (except for the ADOL-Hc1 SHT study where each group contained 900 birds and the UD-3 SVT experiment where there were only 196 ALV-J inoculates and 600 sham inoculates) and placed in a commercial type broiler house that had been divided by a 4-ft wide wire mesh corridor that prevented direct contact between the two groups of chickens but allowed free air flow. Each treatment group was placed at equal bird density (0.72 ft^2 per bird) and had the same drinker and feeder space per bird. Feed, heating, and lighting programs were consistent with commercial standards. The chickens were sent to a commercial processing plant between 49 and 51 days of age, except the UD-3 SVT study where the birds were necropsied at 49 days and condemnations were assessed using standard processing plant inspection criteria.

To simulate ALV-J horizontal transmission post-hatch, one group of birds was inoculated with $10^{3.7}$ mean tissue culture infective dose ($TCID_{50}$) of the appropriate isolate of ALV-J by the intraabdominal (IA) route. The other group, which served as sham inoculated controls, was inoculated with an equal volume of sterile diluent by the IA route.

To simulate commercial field conditions each animal was exposed to approximately 10^2 mean chicken infective dose (CID_{50}) of chicken anemia virus (CAV) Del-Ros strain (8), $10^{3.5}$ EID_{50} infectious bursal disease virus (IBDV) variant E (7), and $10^{3.5}$ EID_{50} of a pathogenic reovirus isolate (2408 isolate) (9,13) at 3, 7, and 10, days of age via drinking water. Both groups of birds were also exposed to a contemporary, very virulent Marek's disease virus (MDV) isolate via virus shedders. MDV shedders were hatch mates of the experimental chickens that were not vaccinated for MDV but were inoculated by the IA route at hatch with MDV. One MDV shedder was placed per 24 chickens in each of the treatment groups at hatch. The MDV shedders were removed prior to processing.

To confirm ALV-J exposure status in the experimental birds, buffy coats and plasma were obtained from 90 uninoculated hatch mates of the experimental birds at hatch and, subsequently, 45 chickens in each treatment group were wing-banded so that the same birds were monitored throughout the experiment and bled at 2, 4, 6, and 8 weeks of age to assess ALV-J viremia by virus isolation from whole blood.

Weekly, 20% of the birds in each treatment group were weighed. Body weights were obtained from all birds approximately 24 hr prior to processing.

Any chickens dying during the course of the experiments were necropsied, and gross and microscopic lesions were recorded. At processing gross lesions were recorded and tissues were collected from condemned birds for microscopic evaluation.

Tissues collected for microscopic evaluation were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned, and stained with hemotoxylin and eosin.

Simulated vertical transmission (SVT) studies. The same three ALV-J isolates evaluated by SHT—ADOL-Hc1, UD-3, and UD-4—were evaluated by SVT. The birds were placed and grown in a fashion identical to that described for the SHT studies with the following exception: ALV-J exposure was accomplished by inoculation of broiler hatching eggs at 3 days of incubation by the yolk sac route with $10^{3.7}$ $TCID_{50}$ per egg of the appropriate isolate of ALV-J (half of the eggs), the other half were sham inoculated with an equal volume of sterile diluent.

Vaccine response. Eighty birds per treatment group from the UD-3 and UD-4 experiments and 40 birds from each treatment group in the ADOL-Hc1 experiments were not sent to processing and were divided into four equal groups. One group from each treatment group (sham or ALV-J inoculated) was challenged with $10^{3.5}$ $TCID_{50}$ of IBV Ark, IBV Mass, NDV (Texas GB strain), or avian reovirus (2408 isolate). IBV and NDV were administered by the intraocular route; reovirus was administered by the footpad route.

IBV protection was evaluated by obtaining tracheal swabs 5 days post infection (PI). Swabs were processed for virus isolation by inoculating 0.2 ml swab tube material into embryonated specific-pathogen free (SPF) eggs at 9 to 10 days of incubation by the chorioallantoic sac route. Eggs were incubated for 7 days and candled daily for viability. Seven days PI the embryos were evaluated for IBV-specific lesions, including stunting and curling.

NDV protection was evaluated by observing the challenge birds for neurological signs or mortality for 10 days PI.

Reovirus protection was assessed by scoring the inoculated footpad for inflammation 3, 4, and 5 days PI.

Statistical analysis. The statistical significance of differences in chicken body weights was evaluated with a two-tailed Student's *t*-test with the SAS software package (SAS Institute Inc., Cary, NC). Mortality, condemnations, and protection data were analyzed by the chi-square test of independence. A P value of ≤ 0.01 was used to define statistical significance.

RESULTS

Total mortality and culls are shown in Table 1. In all six experiments total mortality and culls were higher in the ALV-J inoculates than in sham inoculated controls. The greatest difference between

Table 1. Percent total mortality and culls, disease condemnations, and ALV-J associated tumor incidence (percent of chickens observed with ALV-J associated tumors) for sham and ALV-J inoculated meat-type chickens.

Isolate	Route of exposure	N		Mortality			Disease condemnations			% ALV-J associated tumors	
		Sham	ALV-J	Sham	ALV-J	% Difference in mortality	Sham	ALV-J	% Difference in disease condemnation	Sham	ALV-J
ADOL-Hc1	SHT[A]	900	900	14.1	18.3	4.2	0.4	2.1	1.7*	0	0.11
ADOL-Hc1	SVT[B]	600	600	7.8	15.8	8*[C]	1.4	3.3	1.9	0	0.6
UD-4	SHT	600	600	4.5	6.5	2	0.6	1.0	0.4	0.1	0.95
UD-4	SVT	600	600	7.4	28.6	21.2*	1.0	15.5	14.5*	0.1	3.4
UD-3	SHT	600	600	12	15.5	3.5	0	0.45	0.45	0	2.4
UD-3	SVT	600	196	10.5	35.7	25.2*	2.4	42.8	40.4*	0.1	4.1

[A]SHT = simulated horizontal transmission.
[B]SVT = simulated vertical transmission.
[C]Asterisk denotes statistical significance at a P value of 0.01.

sham and ALV-J inoculates in total mortality and culls was observed in the UD-4 SVT experiment (a 21.2% difference). The least difference was also with the UD-4 isolate, but with SHT (a 2% difference). Mortality was significantly higher in the ALV-J inoculates in all of the SVT experiments ($P = 0.01$).

Morbidity observed during the experiments varied by isolate and inoculation route. Clinical signs were mild or absent in the ADOL-Hc1 SHT study, whereas severe disease characterized by poor feathering, depression, and unevenness was seen in the UD-4 and UD-3 SVT groups. Additionally, experiments with the UD-4 isolate often produced grossly visible myelocytomas in the heads of infected birds and was more prevalent in the SVT study. Other lesions that were often observed at necropsy in both sham and ALV-J inoculates were bacterial infections presented as perihepatitis or omphalitis. Ascites and air sacculitis were also observed in both treatment groups. The number of chickens with these lesions was higher in the ALV-J inoculates as compared to the sham inoculates. Mortality rates of chickens with no visible gross lesions were also increased in the ALV-J inoculates.

Disease condemnations are shown by experiment in Table 1. Disease condemnations were greater in the ALV-J inoculates as compared to the sham inoculates in all experiments. Increased condemnations were also observed in all SVT experiments as compared to SHT studies. Condemnations were significantly higher in the ALV-J inoculates in the ADOL-Hc1 SHT experiment and in the UD-3 and UD-4 SVT experiments. Increases in condemnations were primarily due to skin leukosis and visceral tumors. Microscopic evaluation determined that most of these tumors were MDV induced. Disease condemnations occurred in both ALV-J and sham inoculates and were classified as "air sac," "infectious process," and "septicemia/toxicemia" condemnations.

ALV-J associated tumor incidence is shown in Table 1. The ALV-J tumor incidence was determined by microscopic evaluation of grossly visible tumors in birds that died during the experiment or birds condemned at processing. ALV-J associated tumors were observed in one sham inoculate in three of the six experiments, and tumor incidence in ALV-J inoculates ranged between 0.11% and 4.1% among the experiments. The types of ALV-J associated tumors observed during the study included myelocytomas, nephroblastomas, multicentric hystiocytosis, hemangiomas, and one bile duct carcinoma.

In the five experiments from which feed conver-

Table 2. Feed conversions and mean body weights at processing for ALV-J and sham inoculated chickens.

Isolate	Route of exposure	Feed conversion		Mean body weight at processing (kg)		
		Sham	ALV-J	Sham	ALV-J	% Difference
ADOL-Hc1	SHT[A]	ND[C]	ND	2.65	2.48	6.3[D]
ADOL-Hc1	SVT[B]	2.16	2.27	2.72	2.6	4.4*
UD-4	SHT	1.7	1.96	2.57	2.32	9*
UD-4	SVT	2.02	2.21	2.53	2.17	14*
UD-3	SHT	1.78	2.44	2.27	2.05	9.7*
UD-3	SVT	2.45	4.84	2.66	1.52	42.7*

[A]SHT = simulated horizontal transmission.
[B]SVT = simulated vertical transmission.
[C]ND = not done.
[D]Asterisk denotes statistical significance at a *P* value of 0.01.

sion data was obtained, feed conversions were between 0.11 and 2.39 points higher in the ALV-J inoculates than in the sham inoculated controls (Table 2). The largest changes in feed conversions were seen in the experiments with the UD-3 isolate, where in the SVT study the feed conversion nearly doubled in the ALV-J inoculates. The least effect on feed conversion was observed in the experiments with the ADOL Hc1 isolate.

Mean body weights at processing were decreased between 6.3% and 42.7% in the ALV-J inoculates from the mean body weights of the sham inoculates in all six experiments (Table 2). Decreases in the body weights of ALV-J inoculates were statistically significant at a *P* value of 0.01 at 6 weeks of age and at processing and in the ADOL-Hc1 SHT experiment, and at 1, 2, and 6 weeks of age and at processing in the ADOL-Hc1 experiment. Body weight differences were significantly lower at all sample times except hatch and week two for the UD-4 SHT experiment, and at all sampling times except hatch during the UD-3 and UD-4 SVT experiments. Decreased body weights were only significant from week five onward in the UD-3 SHT study. At hatch in the ADOL-Hc1 SVT study and at 1 week of age during the UD-3 SHT study, body weights in the ALV-J inoculates were significantly higher. The largest body weight differences at processing were seen in the SVT studies with the UD-3 and UD-4 isolates.

The mean body weights of the ALV-J inoculated chickens were significantly lower than the body weights of the sham inoculates at 1 week of age in all but the ADOL-Hc1 SHT and UD-3 SHT studies, in which the ALV-J inoculates' body weights were not significantly lower than those of the sham inoculates until 6 and 5 weeks of age, respectively (Table 2). At hatch in the ADOL-Hc1 SVT experiment and at 1 week of age in the UD-3 SHT experiment, the mean body weights of the ALV-J inoculates were significantly increased above those of the sham inoculates.

The protection of sham and ALV-J inoculates against challenge with IBV Ark or Mass serotypes is summarized in Tables 3 and 4. Owing to unusually high mortality, vaccine response studies could not be performed in the UD-3 SVT experiment. Protection against IBV Ark challenge was between 85% and 100% in both treatment groups and was slightly decreased in the ALV-J exposed chickens in two experiments and increased in the ALV-J inoculates in the ADOL-Hc1 SVT experiment. Protection to IBV Mass was between 72% and 100% and between 61% and 89% in the sham inoculates and ALV-J inoculates, respectively. Protection against IBV Mass was lower in the ALV-J inoculates in all five experiments. Protection against NDV for sham and ALV-J inoculates is shown in Table 5. There was 100% protection against NDV challenge in the sham inoculates in all experiments; however, protection in the ALV-J inoculated groups was 91% or less in three of five experiments. Protection against reovirus footpad challenge was 100% in the sham inoculates in all but the UD-4 SVT experiment, where there was 55% protection (Table 6). The ALV-J inoculates had between 81% and 100% protection to reovirus in all but the UD-4 SVT experiment, where there was only 18% protection. Protection against reovirus was decreased in the ALV-J inoculates as compared to that of the sham inoculates in three of five experiments. There were no statistically significant differences between the treatment groups in any of the protection studies.

Table 3. Protection of sham and ALV-J inoculated chickens against challenge with IBV Ark at 8 weeks of age. Differences in protection were not significant at a P value of 0.01.

ALV-J isolate	ALV-J route of exposure	Sham		ALV-J	
		No. protected/total	% Protected	No. protected/total	% Protected
ADOL-Hc1	SHT[A]	10/10	100	9/9	100
ADOL-Hc1	SVT[B]	9/10	90	11/11	100
UD-4	SHT	15/16	94	15/17	88
UD-4	SVT	19/19	100	18/20	90
UD-3	SHT	17/20	85	17/20	85
UD-3	SVT	ND[C]	ND	ND	ND

[A]SHT = simulated horizontal transmission.
[B]SVT = simulated vertical transmission.
[C]ND = not done.

DISCUSSION

Disease caused by ALV-J is characterized by tumor development, often myeloid leukosis, increased mortality, and decreased body weights (5,6,11). Previously, economic loss from ALV-J induced disease was recognized to be due to tumor associated mortality in breeders, leading to decreased production. Therefore the impact of ALV-J infection on broilers was expected to be minimal due to their short life span. However, field observations suggest that ALV-J infected broiler flocks do not perform as well as uninfected flocks and respond less well to environmental and infectious stressors. Various performance criteria were evaluated in this study to determine the effects of ALV-J on broilers in a simulated commercial setting.

The effects on body weight and feed conversion that were observed in all six experiments have important economic implications. Body weight effects have been previously observed with ALV subgroups C and subgroup J infection in chickens (2,3,11). The mechanism for the effect of ALV-J on body weights and feed conversion is not clear; however, the decreased body weights are not simply due to anorexia from disease as reflected by increased feed conversions. Importantly, during these studies, the chickens were experimentally exposed to several immunosuppressive viruses, including reovirus 2408, a pathogenic isolate that is known to affect body weights (9). Therefore it cannot be ruled out that the body weight and feed conversion effects were in some part due to an interaction between ALV-J and reovirus and/or the other viruses that were present.

The severity of the impact of ALV-J on performance also varied among isolate/breed combinations. The experiments with the UD-3 and UD-4 isolates produced more severe neoplastic and nonneoplastic disease than the experiments with

Table 4. Protection of sham and ALV-J inoculated chickens against challenge with IBV Mass at 8 weeks of age. Differences in protection were not significant at a P value of 0.01.

ALV-J isolate	ALV-J route of exposure	Sham		ALV-J	
		No. protected/total	% Protected	No. protected/total	% Protected
ADOL-Hc1	SHT[A]	10/10	100	8/9	89
ADOL-Hc1	SVT[B]	9/10	90	7/10	70
UD-4	SHT	13/18	72	14/23	61
UD-4	SVT	15/21	72	10/16	63
UD-3	SHT	14/18	78	14/20	70
UD-3	SVT	ND[C]	ND	ND	ND

[A]SHT = simulated horizontal transmission.
[B]SVT = simulated vertical transmission.
[C]ND = not done.

Table 5. Protection of sham and ALV-J inoculated chickens against challenge at 8 weeks of age with NDV (Texas GB strain). Differences in protection were not significant at a P value of 0.01.

ALV-J isolate	ALV-J route of exposure	Sham		ALV-J	
		No. protected/total	% Protected	No. protected/total	% Protected
ADOL-Hc1	SHT[A]	10/10	100	9/9	100
ADOL-Hc1	SVT[B]	11/11	100	10/11	91
UD-4	SHT	18/18	100	18/18	100
UD-4	SVT	19/19	100	13/15	87
UD-3	SHT	20/20	100	17/20	85
UD-3	SVT	ND[C]	ND	ND	ND

[A]SHT = simulated horizontal transmission.
[B]SVT = simulated vertical transmission.
[C]ND = not done.

ADOL-Hc1. Among the isolate/breed combinations there was also a difference in their effects on specific performance criteria. For example, the experiments with the UD-3 isolate had the most severe effects on body weight and feed conversion, and the experiments with the UD-4 isolate produced myelocytomas in the skulls of many of the infected birds, unlike experiments with UD-3 or ADOL-Hc1 where this lesion was not observed. Further studies need to be performed to determine the specific contributions of isolate and host genetics to ALV-J pathogenesis and characteristics of specific isolates within a single genetic line.

Age at exposure also clearly contributed to the severity of ALV-J induced disease. SVT produced more severe disease than SHT in all experiments. Age associated resistance to disease has been found with many viruses and is based on the relative maturity of the immune system at the time of exposure. For example, chickens that are exposed to ALV-J *in ovo* may not be able to mount an immune response as effectively as a day old chick and may, in fact, develop immune tolerance to the virus. Additionally, embryo inoculation may allow for a greater number of cells and cell types to become infected, leading to an increased virus load as compared to chick inoculation.

Interestingly, the increase in mortality and morbidity in the ALV-J infected birds in this study was generally not due to tumors. Conditions characterized by bacterial infections were common, and although they were also seen in the sham inoculates, the number and severity were increased in the ALV-J inoculates. In general, mortality was high in both treatment groups during these studies. Specifically, the reason for high mortality in the ADOL-Hc1 SHT study is believed to be due to a bacterial infection obtained at the hatchery that was subsequently transmitted from chick to chick during the inoculation process. The reason for the high mortality in other experiments in unclear but may be associated in part with increased growth

Table 6. Protection of sham and ALV-J inoculated chickens against avian reovirus (2408 isolate) footpad challenge at 8 weeks of age. Differences in protection were not significant at a P value of 0.01.

ALV-J isolate	ALV-J route of exposure	Sham		ALV-J	
		No. protected/total	% Protected	No. protected/total	% Protected
ADOL-Hc1	SHT[A]	10/10	100	9/9	100
ADOL-Hc1	SVT[B]	11/11	100	9/11	81
UD-4	SHT	20/20	100	13/16	81
UD-4	SVT	11/20	55	3/17	18
UD-3	SHT	20/20	100	20/20	100
UD-3	SVT	ND[C]	ND	ND	ND

[A]SHT = simulated horizontal transmission.
[B]SVT = simulated vertical transmission.
[C]ND = not done.

rates observed in chickens grown at our facilities as compared to a commercial environment.

Increases in disease condemnations were due to both tumors and conditions associated with bacterial infections. Importantly, ALV-J associated tumors were responsible for very few condemnations. Most "leukosis" condemnations were due to MDV induced tumors, of which the number and severity were increased as compared to the sham inoculates. This is consistent with previous studies that demonstrated a synergistic effect between ALV-J and MDV during concomitant infection (14).

Previous studies assessing *in vitro* indicators of immunosuppression revealed no differences in immune function based on exposure to ALV-J (12). However increased mortality, morbidity, and disease condemnations in the ALV-J inoculates suggest that the ability of the ALV-J infected birds to resist infectious diseases may be compromised. Additionally, although marginal and not statistically significant, trends of decreased protection to challenge with IBV, NDV, and reovirus after vaccination or prior exposure is consistent with immunosuppression. More work needs to be done, particularly *in vivo,* to characterize the effect ALV-J on the immune system. The reason why protection to reovirus challenge was substantially reduced in the UD-4 SVT experiment in both ALV-J and sham inoculates as compared to the other experiments is not clear.

Finally, these studies represent the effects of ALV-J infection in a worst-case scenario. In the field, all members of a flock would not be infected at the same age, especially not vertically, nor would transmission be efficient enough to spread horizontally at hatch within an entire flock. One other factor that must be considered is that there was evidence of a low level of ALV infection in the sham inoculates by virus isolation, although the incidence was consistently much lower than in the ALV-J inoculates. The source of the infection was not due to cross-contamination from the ALV-J inoculated chickens, since sham inoculated groups with chickens that were positive for virus isolation contained chickens that were positive for virus at hatch, but is probably due to the animals being from a commercial source (although the source flocks were screened prior to obtaining eggs or chicks). The subgroup of the background infection is not known but is most likely subgroup A or J; therefore, the sham inoculates cannot be regarded as true negative controls, although antibody to ALV subgroups A and B was rarely detected in any of the experimental birds by commercial ELISA (data not shown) and ALV-J antibody was not consistently detected by virus neutralization assay. Regardless of this, the only differences between the ALV-J inoculates and sham inoculates was experimental exposure to ALV-J; therefore, this can be considered a contributing factor for the differences in performance.

The results of this study reveal that there is an adverse impact of ALV-J infection on broiler performance in a simulated commercial environment and that the impact of ALV-J infection is primarily due to nonneoplastic disease. Although not definitive, there are suggestions that ALV-J may be associated with immunosuppression in broiler chickens produced in a commercial environment. The role of ALV-J alone and in combination with other pathogens needs to be better defined.

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ACKNOWLEDGMENTS

The authors would like to thank Marcy Troeber, Bob Alphin, Hillary Reidy, Dr. Spangler Klopp, Keith Moore, and Dr. Don Ritter for technical and other assistance with this study. We also thank Dr. Aly Fadly, for providing the ADOL-Hc1 isolate. These studies were funded by a U.S. Poultry and Egg Association grant, project 435.